

Review

Soy Isoflavones—Benefits and Risks from Nature's Selective Estrogen Receptor Modulators (SERMs)

Kenneth D. R. Setchell, PhD

Department of Pediatrics, Children's Hospital Medical Center, Cincinnati, Ohio

Key words: soy isoflavones, phytoestrogens, pharmacokinetics, phytoprotectants

Phytoestrogens have become one of the more topical areas of interest in clinical nutrition. These non-nutrient bioactive compounds are ubiquitous to the plant kingdom and possess a wide range of biological properties that contribute to the many different health-related benefits reported for soy foods and flaxseeds—two of the most abundant dietary sources of phytoestrogens. Reviewed is the recent knowledge related to their pharmacokinetics and clinical effects, focusing mainly on isoflavones that are found in high concentrations in soy foods. Arguments are made for considering soy isoflavones as natural selective estrogen receptor modulators (SERMs) based upon recent data of their conformational binding to estrogen receptors. Rebuttal is made to several key and important issues related to the recent concerns about the safety of soy and its constituent isoflavones. This article is not intended to be a comprehensive review of the literature but merely highlight recent research with key historical perspectives.

Key teaching points:

- Soy is the richest dietary source of bioactive phytoestrogens called isoflavones, and their bioavailability is highly dependent on intestinal bacterial metabolism.
- Plasma urinary concentrations of isoflavones exceed by several orders of magnitude the levels of endogenous estrogens after consuming relatively modest amounts of soy foods and biological effects can be expected.
- The pharmacokinetic behavior of isoflavones indicates that the maximal health benefits are most likely to be derived by consuming small amounts of isoflavone-rich foods throughout the day.
- Maximal health benefits from phytoestrogen-rich foods are more likely to occur from regular and lifelong consumption.
- Isoflavones have characteristics that are consistent with selective estrogen receptor modulators and not estrogens. As such, when consumed at usual dietary intakes consistent with intakes by Asians, isoflavones are unlikely to have the negative effects associated with estrogens.

INTRODUCTION

In recent years there has been an exponential increase in the number of basic science, clinical and nutritional studies investigating the potential health effects of phytoestrogens, as reviewed in detail elsewhere [1,2]. A range of different classes of phytoestrogens is found in plant-based diets, but most of the clinical and nutritional interest has focused on the lignans that are abundant in flaxseed [3] and the isoflavones that are found in almost all soy protein-containing foods [4]. What may not be appreciated is that the levels of intake of these biologically

active phytoestrogens exceed by several orders of magnitude the levels of intakes of the synthetic endocrine disruptors, classified as xenoestrogens [5]. Typical circulating concentrations of isoflavones can exceed endogenous estradiol concentrations by 10,000- to 20,000-fold in adults [1,6–9] and infants [10] and, as such, can be expected to exert biological effects at the molecular, cellular, or physiologic level. The most pertinent issue of late has been whether such effects are of a beneficial or detrimental nature. In this condensed overview, the basic pharmacology of isoflavones is outlined and a critical review of the arguments centered on the potential effects of phytoestrogens is

Presented in part at Ross Products Research Conference on Medical Issues, "Synergy in Medical and Nutritional Therapy," November 6–8, 2000. Key Largo, Florida. Address reprint requests to: Kenneth D. R. Setchell, PhD, Professor of Pediatrics, Director, Clinical Mass Spectrometry, Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, Ohio 45229. Email: SETCK0@CHMCC.ORG

Journal of the American College of Nutrition, Vol. 20, No. 5, 354S–362S (2001)
Published by the American College of Nutrition

discussed. Commentary is restricted to the isoflavones that comprise the major class of phytoestrogens found in soybeans, clover and the Chinese vine, kudzu. While the latter two plants are not common components of the human food chain, isoflavones extracted from them are nevertheless now incorporated into many commercially available phytoestrogen supplements [11].

PHARMACOKINETICS OF ISOFLAVONES

There is a vast historic literature on the identification and metabolism of isoflavones in a wide range of animals, including sheep [12,13], cow [14], horse [15], fowl [16–18], dogs [19], monkeys [20], and rodents [21–25]. This early interest in phytoestrogens emanates from the finding that isoflavones present in *Trifolium subterraneum* were responsible for an infertility syndrome in sheep that grazed on pastures in S.W. Australia where this species of clover was prevalent [26]. By contrast, data on the pharmacokinetics of isoflavones in humans is sparse [1,11,27–29], and has been slow to emerge even though isoflavones were first identified in the urine of humans in 1980 [30,31]. These early studies showed that soy protein foods are the major source of isoflavones in the human diet [4,31]. These compounds, because they naturally occur in the soybean almost exclusively as polar glycosides [32–37], require intestinal bacteria for their bioavailability [4] and have been shown to undergo a classic enterohepatic circulation [22] common to many steroids, including estrogens [38]. So crucial is the requirement of an intact bacterial flora, that without hydrolysis of the sugar moiety, dietary isoflavones are not bioavailable to any appreciable amount [39]. Several recent studies using a rat intestinal perfusion system and monolayers of human Caco-cells have shown that genistin, the β -glycoside of genistein, does not penetrate the enterocyte to any appreciable degree (references reviewed in [39]). Hydrolysis of the glycosidic bond occurs by the action of β -glucosidases [40] that are presumed to be mainly of bacterial origin judged by the very long time it takes to attain peak plasma concentrations when isoflavone glycosides are ingested orally [11]. Although membrane bound β -glucosidases are present in the intestine [41,42], their role in hydrolysis of isoflavone glycosides must be considered minimal given that these conjugates do not penetrate the enterocyte. There is an established developmental expression of bacterial β -glucosidase activity [43], and clearly sufficient activity in early life to account for hydrolysis of isoflavones found in soy infant formulas [10,44]. This is evident from the very high plasma concentrations seen in infants consuming soy formula [10]. In an extensive number of studies that we have performed with pure isoflavones, [^{13}C]stable-labeled analogues, and isoflavone-rich foods, it is evident that the origins of the isoflavones strongly influence the pharmacokinetics [45].

Pharmacokinetic studies show that after oral ingestion of

individual isoflavones, peak plasma concentrations are attained 5–6 hours later for the aglycones and the clearance from plasma proceeds with a half-life of systemic elimination of 6–8 hours [11]. Notable differences are seen in the pharmacokinetics of daidzein and genistein and their corresponding β -glycosides. Plasma concentrations of genistein are consistently higher than daidzein when equimolar amounts are ingested. This is attributed to the much greater volume of distribution of daidzein compared with genistein and its higher clearance rate. The bioavailability of genistein is higher than that of daidzein and the overall bioavailability of isoflavones when ingested as the β -glycosides is highest when they are ingested as aglycones, as determined following single-bolus oral administration [11]. The rate of absorption of the aglycones is much faster than that of the β -glycosides [11], a finding confirmed in a recent study comparing a fermented and unfermented soy food product [29]. This would be predicted based on chemical structure, because at normal intestinal pH the aglycones will be rapidly absorbed by a process of non-ionic passive diffusion. Our studies show that the time to reach the maximal plasma concentration is significantly longer when the β -glycosides are ingested [11]. However, the aglycones are more vulnerable than the corresponding β -glycosides to further degradation to an array of other metabolites [46,47], thus limiting their bioavailability. This picture is analogous to what is known for the pharmacokinetics of flavonoids [48]. Our studies have also revealed a curvilinear relationship between the systemic bioavailability as measured from the AUC of the plasma concentration curves and amount isoflavones ingested, at least where food is concerned [45]. There occurs a decreased fractional absorption at doses of intake exceeding 0.5 mg/kg body weight, indicating that uptake is saturable and that there is probably no added benefit to consuming large amounts of isoflavones in soy foods. This finding is important with regard to the safety profile of isoflavones in soy foods, and suggests there is no advantage to fortifying foods with high levels of isoflavones as appears the current trend by the food industry. Based on the pharmacokinetics, maximum steady-state plasma levels are more likely to be attained by repeated ingestion throughout the day of several servings of soy foods having modest isoflavones levels. This contention is supported by data from studies of infants fed soy formulas where the plasma levels attained by repeated feeds throughout the day are approximately tenfold higher than those seen in adults consuming similar quantities of isoflavones [10].

Knowledge of the pharmacokinetics, including assessment of bioavailability, is crucial to the design of clinical studies examining efficacy because it cannot be assumed that all soy foods deliver comparable isoflavone bioavailabilities. It should also be pointed out that there is remarkable variability in the isoflavone content of soy foods [34,36,37,49,50] and that even the same product will vary considerably over time. This has been highlighted in a number of previous reports and renders food tables for assessing isoflavone intake of limited value [51,52]. In recent studies we have found that batches of isolated

soy protein produced for the food industry varied over a three-year period by up to 400% in the isoflavones content (Setchell & Cole unpublished data). While there is no requirement on the part of food manufacturers to label their products with the isoflavone content, some are already doing so, and this is helpful. In the absence of this information, the only way to assess intake is to measure the isoflavone levels in the food products, and this is generally accomplished by a number of different HPLC methods [34,36,53,54].

USUAL NUTRITIONAL LEVELS OF ISOFLAVONE INTAKE

The issue of what constitutes normal dietary levels of isoflavone intake, particularly in Asian populations where soy foods are a staple, is controversial. Based on urinary isoflavone excretion, researchers first suggested that typical intakes of isoflavones ranged from 50 to 150 mg/day in Japanese adults [4]. This was later disputed and a more conservative estimate of 50 mg/day was proposed as more likely [55]. There have been no direct attempts to estimate daily intake of phytoestrogens in Japanese adults, although several have used dietary recall to provide estimates of soy food intake in persons living in Japan [56–58], China [59], Indonesia [60], and Japanese-Americans in Hawaii and Canada [56,61]. Nagata *et al.* [57] reported that the average daily amount of soy foods consumed by Japanese adults is 54.4 and 63.6 g for women and men, respectively. However, it should be noted that there was a huge individual variation. This intake of soy foods corresponds to 8.00 g and 6.88 g of total soy protein for women and men, respectively. From these figures, a reasonable assessment of isoflavone intake can be calculated by assuming 2–5 mg isoflavones per gram soy protein (Setchell and Cole, unpublished data). Based on this assumption, Japanese adults probably consume 15–45 mg of isoflavone/day on average. Estimates of isoflavone intakes by Chinese adults are similar [59], while it is evident that Indonesians must ingest much higher levels based on the relatively large quantities of tofu and tempe consumed [60]. Recent published figures show the median value for intake of these foods to be 125 g/day for elderly people living in Jakarta with the range being 62 and 250 g/day for the 25th and 75th percentiles of intake. This is considerable and while there is a wide variability in isoflavone content of tempe and tofu, this study would suggest that intakes in excess of 150 mg/day may be attainable. Interestingly, the incidence of breast cancer and prostate cancer in Indonesia is considerably lower than it is in Japan and China, and would appear to inversely correlate with the isoflavone intake among these three countries. Intakes of isoflavones by Asians are much higher than intakes of Westerners, which are clearly negligible based upon the extremely low concentrations in urine and plasma. According to one report, the usual intake of isoflavones in the British diet is <1 mg/day [62].

ISOFLAVONES, NATURAL SELECTIVE ESTROGEN RECEPTOR MODULATORS (SERMS)

Perhaps the greatest misnomer has been the liberal classification of soy isoflavones as ‘estrogens’. On the one hand, criticism has been leveled at soy isoflavones’ relative ineffectiveness in several clinical studies when compared with the actions of classical estrogen replacement therapy. Yet, paradoxically, concerns have been voiced that soy isoflavones are potentially harmful because they are ‘estrogens,’ albeit natural. These phytoestrogens are in fact non-steroidal in chemical structure. However, due to the presence of the phenolic rings, and particularly the 4’-hydroxyl, they have the ability to bind to estrogen receptors, as do many substances, including antiestrogens like tamoxifen used successfully to treat breast cancer. Isoflavones show a higher relative affinity for binding to the ER β receptor, about six- to eightfold [63], and appear to model on selective estrogen receptor modulators (SERMs) in their conformational binding to the receptor [64]. Elegant X-ray crystallographic studies have probed and compared the conformational binding of estrogens [65], the recently approved SERM raloxifene (marketed in the USA by the name Evista), and the isoflavone genistein [66]. These studies show important and distinct differences in positioning of the compound within the dimerized ER-complex. Most striking is the position of several of the protein helices of the ER that are crucial in determining agonist or antagonist actions. The folding of helix-12 in particular governs the ability of the receptor complex to attract specific co-activator or co-repressor proteins [67,68] and this ultimately dictates the extent of agonist or antagonist actions [65]. In this regard, it has been shown that genistein sits in the ER-complex in a manner that is almost identical to that of raloxifene, and not like estradiol [66]. So, rather than classifying soy isoflavones as ‘estrogens,’ they should more correctly be judged to act hormonally as natural SERMs, as was recently suggested [69]. As such, this suggests that soy isoflavones are likely to have the beneficial effects of estrogen without the negatives, especially in tissues such as the endometrium and breast [64,70].

PHYTOESTROGENS AND THEIR CLINICAL IMPLICATIONS

The low incidence of hormone-related diseases in countries where soy foods are regularly consumed is what originally stimulated interest in this class of phytoprotectants [4]. Numerous dietary intervention studies using soy foods containing isoflavones have been performed, mostly in areas related to cholesterol-lowering and cardiovascular disease [71]. The US Food and Drug Administration recently approved a health claim for soy protein reducing the risk for heart disease because of compelling evidence, reviewed partly in a meta-analysis by

Anderson *et al.* [71], that this phytoestrogen-rich food is hypocholesterolemic. However, a role for isoflavones in the cholesterol-lowering response was not recognized by the FDA because of a paucity in supporting data. Yet, animal studies pioneered by groups at Wake Forest University convincingly show that isoflavones contribute to cholesterol-lowering [72], reduction in atheroma [73] and improvement in vascular reactivity [74]. These studies led to numerous reinvestigations of the long known hypocholesterolemic effect of soy protein in humans with the objective of better understanding the mechanism and, more specifically, delineating the contribution of isoflavones. Crouse *et al.* [75] demonstrated an impressive dose-response relationship between the extent of reduction in plasma LDL-cholesterol and dose of isoflavone when soy protein levels were maintained constant at 25 g per day, the level approved by the FDA in the health claim. The effect was more apparent in those subjects with LDL-cholesterol levels above 4.24 mmol/L or 164 mg/dL. Critics opposed to the health claim approval have highlighted the failure of soy protein to lower cholesterol in many people with normal or mildly elevated cholesterol levels. This in fact is not totally true, and although the cholesterol-lowering effect is variable among individuals, several metabolic studies have shown that soy protein with isoflavones can lower blood cholesterol in normocholesterolemic people [76–79].

The real potential of soy foods containing isoflavones is, in this author's opinion, most likely to be in the prevention of heart disease rather than in its treatment, as implied by the wording of the health claim. Amounts considerably less than the recommended 25 g soy protein/day are likely to be helpful in this regard. Soy protein intake by Japanese adults averages about 6–8 g/day and it was found that serum cholesterol levels in adults are inversely correlated with soy protein intake [57]. Although several studies have failed to demonstrate any cholesterol-lowering effects of isoflavones when administered as supplements [80–82], their non-hormonal properties may be of greater significance in reducing risk for heart disease. Several clinical studies have shown that isoflavones reduce the susceptibility of lipids to oxidation [83–87] and they have been recently found to have digitalis-like effects in relaxing coronary arteries by a mechanism that involves antagonism of calcium channels [88]. The anti-inflammatory properties of isoflavones in epithelial cells [89,90] may also be important in protecting blood vessels.

Many studies have also investigated the potential of soy isoflavones to have hormonal-like actions. Their potential value in alleviating the vasomotor effects of menopause has driven sales of many isoflavone supplements. The response in terms of reduction in severity and frequency of hot flashes has been variable and modest. It is evident that phytoestrogens cannot compete with standard estrogen replacement therapy for effectiveness in the relief of these symptoms. Perhaps the most promising application may be the effects of isoflavones on bone [91–95]. Two studies have found that isoflavone-rich

foods reduce bone turnover as measured by changes in surrogate markers of osteoblast and osteoclast activity [86,96]. The only published long-term study in which bone density was measured found impressive effects on limiting postmenopausal osteoporosis [97]. A recent 6-month study also found favorable effects of isolated soy protein on lumbar spine bone mineral density [98]. This remains an important area for further investigation, especially in view of the recent indication that a high soy protein intake was associated with higher bone mineral density in postmenopausal Japanese women [99].

SAFETY ISSUES RELATED TO SOY ISOFLAVONES

The safety of soy foods and their constituent isoflavones has been questioned [100,101], even though these foods have been consumed for a very long time by people in Asia, and by vegetarians. Driving these concerns are data from many animal studies in which high levels of isoflavones have been shown to cause various reproductive problems. However, due consideration to species differences in the metabolism of isoflavones has largely been ignored when extrapolations have been made to humans. For example, soymeal fed to captive cheetah in North American zoos led to infertility and venoocclusive disease [102]. Yet, the fact that cheetah, and many feline species lack UDP-glucuronyltransferases that conjugate steroid hormones and isoflavones, has been disregarded. Isoflavones are extremely potent in this species. Clover disease in sheep [26] was the result of continual ingestion of vast amounts of isoflavones from clover and plasma concentrations were far in excess of those typically found in humans consuming soy foods. Many studies have examined the effects of the phytoestrogens, coumestrol, zearalenone, and genistein, in rodents [103–105]. Humans rarely consume coumestrol in the diet. Zearalenone is a mycotoxin, and when soy protein with isoflavones is fed to rats and mice, it is the more potent isoflavone equol that is the naturally occurring major circulating isoflavone found [106]. The validity of performing experiments with high levels of genistein in rodents is therefore questionable, although of academic interest. Generally unappreciated is the fact that most commercial feed used to breed and raise rodents contains isoflavones from soymeal that is added for its protein quality [106,107]. Our recent studies show that rats and mice are typically exposed multigenerationally to doses of isoflavones in the range 90–150 mg/kg body wt, far higher than the doses consumed by humans habitually consuming soy foods (0.5–1.5 mg/kg body wt). Plasma concentrations of isoflavones in rodents are 30,000- to 60,000-fold higher than estradiol concentrations, yet veterinarian and animal husbandry establishments do not appear to experience overt problems in breeding rodents under these conditions. Of more relevance is the probability that very high levels of intakes of isoflavones from commercial rodent diets may subtly skew estrogen-sensitive experimental

end-points, and that the implications are perhaps greater for experiments investigating the regulation of non-hormonal pathways, and particularly gene expression. More recent studies show that genistein is capable of activating CFTR chloride channels in the genetic disease of cystic fibrosis [108], and regulating the intranuclear trafficking of Akt and forkhead protein in cardiomyocytes [109], both being of potential clinical benefit.

For humans, the potency of soy isoflavones has raised concerns regarding the possibility that phytoestrogens may be a double-edged sword. On the one-hand, phytoestrogens may offer benefits to some groups, while perhaps creating risks to others. Speculation that isoflavones cause thyroid disease has been based on studies showing that genistein is capable of inhibiting thyroid peroxidase (TPO), a key enzyme in the production of thyroid hormones [110,111]. These *in vitro* studies show that the potency of genistein is similar to that for a number of flavonoids that were also tested [110], and the latter group of phytoprotectants are found in abundance in fruits and vegetables. Indeed, dietary intake of flavonoids is estimated to be similar to that of isoflavones when soy foods are consumed [112]. The IC_{50} value for inhibition of TPO by daidzein and genistein was reported as 2.0 and 8.8 μ M, respectively, and this is several orders of magnitude higher than the circulating plasma concentrations of free daidzein (approx. 10 nM [8]) and genistein (approx. 18 nM [11]) when usual dietary intakes of isoflavones are consumed. Thyroid hormone plays a key role in development and many studies have compared growth and development of infants fed soy formulas with those breast-fed [113,114]. No significant differences have been observed, although older soy formulas were not optimally formulated and did compromise development and growth. It is difficult to find case reports of adverse effects, either short-term or long-term, due to soy infant formula use [115]. A recent study of a cohort of 952 adults who had been fed either soy formula or cow-milk formula as infants found no significant differences between the groups, either males or females, with regard to height, weight, BMI, indices of pubertal maturation and numerous other reproductive outcomes, including infertility, and cancer [116].

Finally and perhaps the most legitimate concern is the question of whether a woman with breast cancer should be advised to avoid soy foods and phytoestrogen supplements. Recommendations to avoid soy foods now being given by many health professionals to these patients are not based on any clinical evidence to support this advice. As discussed above, the fact that an isoflavone like genistein acts more like a SERM than an estrogen should be the basis for believing that soy foods are more likely to be beneficial for breast cancer treatment and prevention. The MORE trial of raloxifene supports this view [70]. However, clinical studies are warranted to clarify this important issue with regard to soy and its isoflavones. It should be remembered that following the discovery of high levels of isoflavones in urine and later plasma of women consuming soy protein, it was hypothesized that these naturally occurring

non-steroidal estrogens would be beneficial in the prevention and treatment of breast cancer [4]. There is no evidence to the contrary, and considerable data from *in vitro* and *in vivo* animal studies of breast cancer models [117–121] support this original hypothesis.

REFERENCES

1. Setchell KDR: Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr* 68:1333S–1346S, 1998.
2. Setchell KDR, Cassidy A: Dietary isoflavones: biological effects and relevance to human health. *J Nutr* 129:758S–767S, 1999.
3. Setchell KDR, Lawson AM, Mitchell FL, Adlercreutz H, Kirk DN, Axelson M: Lignans in man and in animal species. *Nature* 287:740–742, 1980.
4. Setchell KDR, Borriello SP, Hulme P, Kirk DN, Axelson M: Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *Am J Clin Nutr* 40:569–578, 1984.
5. Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL: Assessing environmental chemicals for estrogenicity using a combination of *in vitro* and *in vivo* assays. *Environ Health Perspect* 104:1296–1300, 1996.
6. Adlercreutz H, Markkanen H, Watanabe S: Plasma concentrations of phyto-oestrogens in Japanese men. *Lancet* 342:1209–1210, 1993.
7. Morton MS, Wilcox G, Wahlqvist ML, Griffiths K: Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. *J Endocrinol* 142:251–259, 1994.
8. Lapcik O, Hampl R, al-Maharik N, Salakka A, Wahala K, Adlercreutz H: A novel radioimmunoassay for daidzein. *Steroids* 62: 315–320, 1997.
9. Zhang Y, Wang GJ, Song TT, Murphy PA, Hendrich S: Urinary disposition of the soybean isoflavones daidzein, genistein and glycitein differs among humans with moderate fecal isoflavone degradation activity. *J Nutr* 129:957–962, 1999.
10. Setchell KDR, Zimmer-Nechemias L, Cai J, Heubi JE: Exposure of infants to phyto-oestrogens from soy-based infant formula [see comments]. *Lancet* 350:23–27, 1997.
11. Setchell KDR, Brown NM, Desai P, Zimmer-Nechemias L, Wolfe BE, Brashear WT, Kirscher AS, Cassidy A, Heubi J: Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J Nutr* 131: 1362S–1375S, 2001.
12. Braden A, Hart N, Lamberton J: The estrogenic activity and metabolism of certain isoflavones in sheep. *Aust J Agric Res* 18:335–348, 1967.
13. Lindsay DR, Kelly RW: The metabolism of phyto-oestrogens in sheep. *Aust Vet J* 46:219–222, 1970.
14. Lundh T-O, Pettersson H, Keissling K-H: Demethylation and conjugation of formononetin and daidzein in sheep and cow liver microsomes. *J Agric Food Chem* 36:22–25, 1988.
15. Marrian G, Haslewood G: Equol, a new inactive phenol isolated from the ketohydroxyoestrin fraction of mares urine. *Biochem J* 26:1227–1232, 1932.

16. Common R, Aimsworth L: Identification of equol in the urine of the domestic fowl. *Biochim Biophys Acta* 53:403–404, 1961.
17. Cayen MN, Carter AL, Common RH: The conversion of genistein to equol in the fowl. *Biochimica et Biophys Acta* 86:56–64, 1964.
18. Tang G, Common RH: Urinary conversion products of certain orally administered isoflavones in the fowl. *Biochim Biophys Acta* 158:402–413, 1968.
19. Juniewicz PE, Pallante Morell S, Moser A, Ewing LL: Identification of phytoestrogens in the urine of male dogs. *J Steroid Biochem* 31:987–994, 1988.
20. Monfort SL, Thompson MA, Czekala NM, Kasman LH, Shackleton CH, Lasley BL: Identification of a non-steroidal estrogen, equol, in the urine of pregnant macaques: correlation with steroidal estrogen excretion. *J Steroid Biochem* 20:869–876, 1984.
21. Nilsson A: Demethylation of the plant oestrogen bichanin A in the rat. *Nature* 92:358, 1961.
22. Sfakianos J, Coward L, Kirk M, Barnes S: Intestinal uptake and biliary excretion of the isoflavone genistein in rats. *J Nutr* 127:1260–1268, 1997.
23. King RA, Broadbent JL, Head RJ: Absorption and excretion of the soy isoflavone genistein in rats. *J Nutr* 126:176–182, 1996.
24. Coldham NG, Howells LC, Santi A, Montesissa C, Langlais C, King LK, Macpherson DD, Sauer MJ: Biotransformation of genistein in the rat: elucidation of metabolite structure by product ion mass fragmentology. *J Steroid Biochem Mol Biol* 70:169–184, 1999.
25. Coldham NG, Sauer MJ: Pharmacokinetics of [¹⁴C]genistein in the rat: Gender-related differences, potential mechanisms of biological action, and implications for human health. *Toxicol Appl Pharmacol* 164:206–215, 2000.
26. Bennetts HW, Underwood EJ, Shier FL: A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Aust J Agric Res* 22:131–138, 1946.
27. King RA, Bursill DB: Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. *Am J Clin Nutr* 67:867–872, 1998.
28. Xu X, Wang HJ, Murphy PA, Cook L, Hendrich S: Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *J Nutr* 124:825–832, 1994.
29. Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, Kataoka S, Kubota Y, Kikuchi M: Soy isoflavone aglycones are absorbed faster and in higher amounts than their glycosides in humans. *J Nutr* 130:1695–1699, 2000.
30. Axelson M, Kirk DN, Farrant RD, Cooley G, Lawson AM, Setchell KD: The identification of the weak oestrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)chroman] in human urine. *Biochem J* 201:353–357, 1982.
31. Axelson M, Sjøvall J, Gustafsson BE, Setchell KDR: Soya—a dietary source of the non-steroidal oestrogen equol in man and animals. *J Endocrinol* 102:49–56, 1984.
32. Walz E: Isoflavone: a Saponin glucoside in soya. *Justus Liebigs Ann Chem* 489:118–155, 1931.
33. Walter E: Genistein (an isoflavone glucoside) and its aglucone, genistin, from soybeans. *J Amer Oil Chem Soc* 63:3273–3276, 1941.
34. Murphy PA: Phytoestrogen content of processed soybean products. *Food Technol* 43:60–64, 1982.
35. Farmakalidis E, Murphy P: Isolation of 6''-O-acetyldaidzein and 6''-O-acetylgenistein from toasted defatted soy flakes. *J Agric Food Chem* 33:385–389, 1985.
36. Coward L, Barnes NC, Setchell KDR, Barnes S: Genistein and daidzein, and their β -glycosides conjugates: anti-tumor isoflavones in soybean foods from American and Asian diets. *J Agric Food Chem* 41:1961–1967, 1993.
37. Song T, Barua K, Buseman G, Murphy PA: Soy isoflavone analysis: quality control and a new internal standard. *Am J Clin Nutr* 68:1474S–1479S, 1998.
38. Adlercreutz H, Martin F: Biliary excretion and intestinal metabolism of progesterone and estrogens in man. *J Steroid Biochem* 13:231–244, 1980.
39. Setchell KDR, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe B, Kirschner AS, Cassidy A, Heubi JE: Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am J Clin Nutr*, in press, 2001.
40. Day AJ, DuPont MS, Ridley S, Rhodes M, Rhodes MJ, Morgan MR, Williamson G: Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Lett* 436:71–75, 1998.
41. Ioku K, Pongpiriyadacha Y, Konishi Y, Takei Y, Nakatani N, Terao J: beta-Glucosidase activity in the rat small intestine toward quercetin monoglucosides. *Biosci Biotechnol Biochem* 62:1428–1431, 1998.
42. McMahon LG, Nakano H, Levy MD, Gregory JF, 3rd: Cytosolic pyridoxine-beta-D-glucoside hydrolase from porcine jejunal mucosa. Purification, properties, and comparison with broad specificity beta-glucosidase. *J Biol Chem* 272:32025–32033, 1997.
43. Mykkanen H, Tikka J, Pitkanen T, Hanninen O: Fecal bacterial enzyme activities in infants increase with age and adoption of adult-type diet. *J Pediatr Gastroenterol Nutr* 25:312–316, 1997.
44. Setchell KDR, Welsh M, Lim C: HPLC analysis of phytoestrogens in soy protein preparations with ultraviolet, electrochemical, and thermospray mass spectrometric detection. *J Chromatogr* 385:267–274, 1987.
45. Setchell KDR: Absorption and metabolism of soy isoflavones—from food to dietary supplements and adults to infants. *J Nutr* 130:654S–655S, 2000.
46. Kelly GE, Nelson C, Waring MA, Joannou GE, Reeder AY: Metabolites of dietary (soya) isoflavones in human urine. *Clin Chim Acta* 223:9–22, 1993.
47. Joannou GE, Kelly GE, Reeder AY, Waring M, Nelson C: A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavonoids. *J Steroid Biochem Mol Biol* 54:167–184, 1995.
48. Hollman PC, van Trijp JM, Mengelers MJ, de Vries JH, Katan MB: Bioavailability of the dietary antioxidant flavonol quercetin in man. *Cancer Lett* 114:139–140, 1997.
49. Dwyer JT, Goldin BR, Saul N, Gualtieri L, Barakat S, Adlercreutz H: Tofu and soy drinks contain phytoestrogens [see comments]. *J Am Diet Assoc* 94:739–743, 1994.
50. King R, Bignell C: Concentrations of isoflavone phytoestrogens and glucosides in Australian soya beans and soya foods. *Aust J Nutr Diet* 57:70–78, 2000.
51. Reinli K, Block G: Phytoestrogen content of foods—A compendium of literature values. *Nutr Cancer* 26:123–148, 1996.
52. Pillow P, Duphorne C, Chang S, Contois J, Strom S, Spitz M,

- Hursting S: Development of a database for assessing dietary phytoestrogen intake. *Nutr Cancer* 33:3–19, 1999.
53. Lu L-J, Broemelin L, Marshall M, Sadagopa Ramanujam V: A simplified method to quantify isoflavones in commercial soybean diets and human urine after legume consumption. *Cancer Epidemiol Biomarkers Prev* 4:497–503, 1995.
 54. Franke AA, Custer LJ, Wang W, Shi CY: HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids. *Proc Soc Exp Biol Med* 217:263–273, 1998.
 55. Messina M: Isoflavone intakes by Japanese were overestimated. *Am J Clin Nutr* 62:645, 1995.
 56. Wilcox B, Fuchigami K, Wilcox D, Kendall C, Suzuki M, Todoriki H, DJA J: Isoflavone intake in Japanese and Japanese-Canadians. *Am J Clin Nutr* 61:901 (Abstract), 1995.
 57. Nagata C, Takatsuka N, Kurisu Y, Shimizu H: Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. *J Nutr* 128:209–213, 1998.
 58. Wakai K, Egami I, Kato K, Kawamura T, Tamakoshi A, Y L, Nakayama T, Wada M, Ohno Y: Dietary intake and sources of isoflavones among Japanese. *Nutr Cancer* 33:139–145, 1999.
 59. Chen Z, Zheng W, Custer LJ, Dai Q, Shu XO, Jin F, Franke AA: Usual dietary consumption of soy foods and its correlation with the excretion rate of isoflavonoids in overnight urine samples among Chinese women in Shanghai. *Nutr Cancer* 33:82–87, 1999.
 60. Purba Mb, Lukito W, Wahlqvist ML, Kouris-Blazos A, Hadisaputro S, Lestiani L, Wattanapenpaiboon N, Kamsu S: Food intake and eating patterns of Indonesian elderly before the 1998 economic crisis. *Asia Pacific J Clin Nutr* 8:200–206, 1999.
 61. Maskarinec G, Singh S, Meng L, Franke AA: Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population. *Cancer Epidemiol Biomarkers Prev* 7:613–619, 1998.
 62. Jones A, Price K, Fenwick G: Development and application of high-performance liquid chromatographic method for the analysis of phytoestrogens. *J Sci Food Agric* 46:357–364, 1989.
 63. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA: Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 139:4252–4263, 1998.
 64. Jordan VC, Morrow M: Tamoxifen, raloxifene, and the prevention of breast cancer. *Endocr Rev* 20:253–278, 1999.
 65. Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, Carlquist M: Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* 389:753–758, 1997.
 66. Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, Ljunggren J, Gustafsson JA, Carlquist M: Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *Embo J* 18:4608–4618, 1999.
 67. McKenna N, Xu J, Nawaz Z, Tsai S, Tsai M-J, O'Malley B: Nuclear receptor coactivators: multiple complexes, multiple functions. *J Steroid Biochem Mol Biol* 69:3–12, 1999.
 68. Klinge C: Estrogen receptor interaction with co-activators and co-repressors. *Steroids* 65:227–251, 2000.
 69. Brzezinski A, Debi A: Phytoestrogens: the “natural” selective estrogen receptor modulators? *European J Obstetrics Gynecol Reprod Biol* 85:47–51, 1999.
 70. Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, Norton L, Nickelsen T, Bjarnason NH, Morrow M, Lippman ME, Black D, Glusman JE, Costa A, Jordan VC: The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation [see comments]. *JAMA* 281:2189–2197, 1999.
 71. Anderson JW, Johnstone BM, Cook-Newell ME: Meta-analysis of the effects of soy protein intake on serum lipids [see comments]. *N Engl J Med* 333:276–282, 1995.
 72. Anthony M, Clarkson T, Hughes C: Plant and mammalian estrogen effects on plasma lipids of female monkeys. *Circulation* 90:1–235, 1994.
 73. Anthony MS, Clarkson TB: Comparison of soy phytoestrogens and conjugated equine estrogens on atherosclerosis progression in post-menopausal monkeys. *Circulation* 97:829 (Abstract), 1998.
 74. Honore EK, Williams JK, Anthony MS, Clarkson TB: Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. *Fertil Steril* 67:148–154, 1997.
 75. Crouse JR, 3rd, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL: A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. *Arch Intern Med* 159:2070–2076, 1999.
 76. Cassidy A, Bingham S, Setchell KDR: Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women [see comments]. *Am J Clin Nutr* 60:333–340, 1994.
 77. Wong W, O'Brian Smith E, Stuff J, Hachey D, Heird W, Pownell H: Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. *Am J Clin Nutr* 68:1385S–1389S, 1998.
 78. Merz-Demlow B, Duncan A, Wangen K, Xu X, Carr T, Phipps W, Kurzer M: Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *Am J Clin Nutr* 71:1462–1469, 1999.
 79. Wangen K, Duncan A, Xu X, Kurzer M: Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. *Am J Clin Nutr* 73:225–231, 2000.
 80. Nestel PJ, Yamashita T, Sasahara T, Pomeroy S, Dart A, Komesaroff P, Owen A, Abbey M: Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. *Arterioscl Thromb Vascul Biol* 17:3392–3398, 1997.
 81. Hodgson JM, Puddey IB, Beilin LJ, Mori TA, Croft KD: Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans. *J Nutr* 128:728–732, 1998.
 82. Nestel PJ, Pomeroy S, Kay S, Komesaroff P, Behrsing J, Cameron JD, West L: Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. *J Clin Endocrinol Metab* 84:895–898, 1999.
 83. Kapiotis S, Hermann M, Held I, Seelos C, Ehringer H, Gmeiner BM: Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage

- by atherogenic LDL. *Arterioscl Thromb Vasc Biol* 17:2868–2874, 1997.
84. Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H: Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. *Proc Natl Acad Sci U S A* 95:3106–3110, 1998.
 85. Jenkins DJA, Kendall CWC, Garsetti M, Rosenberg-Zand RS, Jackson C-J, Agarwal S, Rao AV, Diamandis EP, Parker T, Faulkner D, Vuksan V, Vidgen E: Effect of soy protein foods on low-density lipoprotein oxidation and ex vivo sex hormone receptor activity—A controlled crossover trial. *Metabolism* 49: 537–543, 2000.
 86. Scheiber MD, Liu JH, Subbiah MTR, Rebar RW, Setchell KDR: Dietary soy supplementation reduces LDL oxidation and bone turnover in healthy post-menopausal women. *Menopause*, in press, 2001.
 87. Wiseman H, O'Reilly J, Adlercreutz H, Mallet A, Bowey E, Rowland I: Isoflavone phytoestrogens consumed in soy decrease F₂-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. *Am J Clin Nutr* 72:395–400, 2000.
 88. Figtree GA, Griffiths H, Lu Y-Q, Webb CM, MacLeod K, Collins P: Plant-derived estrogens relax coronary arteries in vitro by a calcium antagonistic mechanism. *Journal of American College of Cardiology* 35:1977–1985, 2000.
 89. Sadowska-Krowicka H, Mannick EE, Oliver PD, Sandoval M, Zhang XJ, Eloby-Childress S, Clark DA, Miller MJ: Genistein and gut inflammation: role of nitric oxide. *Proc Soc Exp Biol Med* 217:351–357, 1998.
 90. Salzman A, Preiser J-C, Setchell K, Szabo C: Isoflavone-mediated inhibition of tyrosine kinase: a novel anti-inflammatory approach. *J Medicinal Food* 2:179–181, 1999.
 91. Blair HC, Jordan SE, Peterson TG, Barnes S: Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. *J Cell Biochem* 61:629–637, 1996.
 92. Arjmandi BH, Alekel L, Hollis BW, Amin D, Stacewicz-Sapuntzakis M, Guo P, Kukreja SC: Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. *J Nutr* 126:161–167, 1996.
 93. Draper CR, Edell MJ, Dick IM, Randall AG, Martin GB, Prince RL: Phytoestrogens reduce bone loss and bone resorption in oophorectomized rats. *J Nutr* 127:1795–1799, 1997.
 94. Anderson JJ, Ambrose WW, Garner SC: Biphasic effects of genistein on bone tissue in the ovariectomized, lactating rat model. *Proc Soc Exp Biol Med* 217:345–350, 1998.
 95. Ishida H, Uesugi T, Hirai K, Toda T, Nukaya H, Yokotsuka K, Tsuji K: Preventive effects of the plant isoflavones, daidzin and genistin, on bone loss in ovariectomized rats fed a calcium-deficient diet. *Biol Pharm Bull* 21:62–66, 1998.
 96. Brzezinski A, Adlercreutz H, Shaoul Rea: Short-term effects of phytoestrogen-rich diet on postmenopausal women. *Menopause* 4:89–94, 1997.
 97. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW, Jr.: Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr* 68: 1375S–1379S, 1998.
 98. Alekel L, St Germain A, Peterson C, Hanson K, Stewart J, Toda T: Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. *Am J Clin Nutr* 72:844–852, 2000.
 99. Horiuchi T, Onouchi T, Takahashi M, Ito H, Orimo H: Effect of soy protein on bone metabolism in postmenopausal women. *Osteoporosis* 11:721–724, 2000.
 100. Barrett J: Phytoestrogens. Friends or foes? [news]. *Environ Health Perspect* 104:478–482, 1996.
 101. Sheehan DM: Isoflavone content of breast milk and soy formulas: benefits and risks [letter; comment]. *Clin Chem* 43:850; discussion 852, 1997.
 102. Setchell KDR, Gosselin SJ, Welsh MB, Johnston JO, Balistreri WF, Kramer LW, Dresser BL, Tarr MJ: Dietary estrogens—a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology* 93:225–233, 1987.
 103. Faber K, Hughes C: The effect of neonatal exposure to diethylstilbestrol, genistein, and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. *Biol Reprod* 45:649–653, 1991.
 104. Whitten PL, Lewis C, Russell E, Naftolin F: Potential adverse effects of phytoestrogens. *J Nutr* 125:771S–776S, 1995.
 105. Medlock KL, Branham WS, Sheehan DM: The effects of phytoestrogens on neonatal rat uterine growth and development. *Proc Soc Exp Biol Med* 208:307–313, 1995.
 106. Brown NM, Setchell KDR: Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. *Lab Invest*, 81:735–747, 2001.
 107. Thigpen JE, Setchell KD, Ahlmark KB, Locklear J, Spahr T, Caviness GF, Goelz MF, Haseman JK, Newbold RR, Forsythe DB: Phytoestrogen content of purified, open- and closed-formula laboratory animal diets. *Lab Anim Sci* 49:530–536, 1999.
 108. Wang F, Zeltwanger S, Hu S, Hwang T-C: Deletion of phenylalanine 508 causes attenuated phosphorylation-dependent activation of CFTR chloride channels. *J Physiol* 524:637–648, 2000.
 109. Camper-Kirby DC, Welch S, Walker A, Shiraiishi I, Setchell KDR, Schaefer E, Kajstura J, Anversa P, Sussman M: Myocardial Akt activation and gender: nuclear activity in females versus males. *Circ Res*, 88:1020–1027, 2001.
 110. Divi RL, Doerge DR: Inhibition of thyroid peroxidase by dietary flavonoids. *Chem Res Toxicol* 9:16–23, 1996.
 111. Divi RL, Chang HC, Doerge DR: Anti-thyroid isoflavones from soybean: isolation, characterization, and mechanisms of action. *Biochem Pharmacol* 54:1087–1096, 1997.
 112. Hertog MG, Hollman PC, Katan MB, Kromhout D: Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr Cancer* 20:21–29, 1993.
 113. Churella H, Borschel M, Thomas R, Breen M, Jacobs M: Growth and protein status of term infants fed soy formulas differing in protein content. *J Am Coll Nutr* 13:262–267, 1994.
 114. Lasekan JB, Ostrom KM, Jacobs JR, Blatter MM, Ndife LI, Gooch WM, 3rd, Cho S: Growth of newborn, term infants fed soy formulas for 1 year. *Clin Pediatr (Phila)* 38:563–571, 1999.
 115. Setchell KDR, Radd S: Soy and other legumes: Bean around a long time but are they the 'Superfood' of the millenium and what are the safety issues for their constituent phytoestrogens. *Asia Pacific J Clin Nutr* 9:S13–S22, 2000.
 116. Strom BL, Schinnar R, Zeigler EE, Bamhart KT, Sammel MD, Macones GA, Stalings VA, Drulis JM, Nelson SE, Hanson SA:

- Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood. *JAMA* 286:807–814, 2001.
117. Barnes S: Effect of genistein on in vitro and in vivo models of cancer. *J Nutr* 125:777S–783S, 1995.
 118. Barnes S, Grubbs C, Setchell KDR, Carlson J: Soybeans inhibit mammary tumors in models of breast cancer. *Prog Clin Biol Res* 347:239–253, 1990.
 119. Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hamalainen E, Wahala K, Makela T, Hase T: Dietary phytoestrogens and cancer: in vitro and in vivo studies. *J Steroid Biochem Mol Biol* 41:331–337, 1992.
 120. Lamartiniere CA, Moore JB, Brown NM, Thompson R, Hardin MJ, Barnes S: Genistein suppresses mammary cancer in rats. *Carcinogenesis* 16:2833–2840, 1995.
 121. Constantinou AI, Krygier AE, Mehta RR: Genistein induces maturation of cultured human breast cancer cells and prevents tumor growth in nude mice. *Am J Clin Nutr* 68:1426S–1430S, 1998.

Received April 26, 2001.